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Animal Inhalation Studies on Ammonia, Ethylene Glycol, Formaldehyde, Dimethylamine, and Ethanol[1]

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Animal Inhalation Studies on Ammonia, Ethylene Glycol, Formaldehyde, Dimethylamine, and Ethanol. COON, R. A., JONES, R. A., JENKINS, L. J., JR., and SIEGEL, J. (1970). *Toxicol. Appl. Pharmacol.* **16**, 646–655. Ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol were tested for toxic effects by the inhalation route in rats, guinea pigs, rabbits, monkeys, and dogs during continuous or repeated exposures.

Repeated exposures at 155 mg/m^3 of ammonia produced no adverse effects; at 770 mg/m^3 there was ocular and nasal irritation in rabbits and dogs and nonspecific inflammatory changes in the lungs of rats and guinea pigs. Following continuous exposures at 40 mg/m^3, nonspecific inflammatory changes were noted in the lungs; at 127 and 262 mg/m^3 similar changes were seen in the lungs and kidneys. Continuous exposures at levels of 455 and 470 mg/m^3 caused 90–98% mortality in rats, and marked eye irritation in rabbits and dogs.

Animals in repeated ethylene glycol exposures at 10 and 57 mg/m^3 showed no changes that were considered to be chemically induced. Continuous exposure at 12 mg/m^3 resulted in moderate to severe eye irritation in rabbits and corneal damage with apparent blindness in 2 of 15 rats after 8 days.

Animals continuously exposed to 4.6 mg/m^3 formaldehyde, 9 mg/m^3 dimethylamine, and 86 mg/m^3 ethanol showed only mild inflammatory changes, primarily in the lungs.

During the course of investigations of the toxicity of trace contaminants over the past 5 years, a number of studies were carried out on selected materials. The purpose of these studies was to develop background information, sometimes very limited in scope because of the real time situation, which might be helpful in the establishment or support of confined space or industrial guidelines. It was felt that these initial studies might also serve as a baseline for other investigators engaged in inhalation studies.

In the present study animals were exposed to ammonia, ethylene glycol, formaldehyde, dimethylamine, or ethanol for 90 days continuously, and to ammonia or ethylene glycol for 8 hours per day, 5 days per week, for 6 weeks.

[1] The opinions expressed herein are those of the authors and do not necessarily reflect the views of the Navy Department or the naval service at large. The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council, Washington, D.C.

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METHODS

Materials

The materials used were as follows: ammonium hydroxide[2] (ACS grade); gaseous ammonia and dimethylamine contained in pressurized cylinders and having minimum purities of 99%; ethylene glycol (reagent grade); and absolute ethanol (USP grade). Formaldehyde was prepared by adding paraformaldehyde (USP grade) to hot distilled water in quantity to yield a 1.35% solution.

Exposure System

The modified Rochester-type inhalation chambers used in these studies have been previously described by Fultyn (1961). The rate of air flow was maintained at 1.2 m^3/min, the relative humidity at approximately 50%, the temperature at 77 ± 2°F, and the negative pressure at approximately 2.0 inches of water. The "down time" necessary for feeding the animals and servicing the chambers during the continuous exposures was less than 2.2% of the total exposure time.

The general method of contaminant generation of ethylene glycol, formaldehyde, and ethanol consisted of pumping the material from a reservoir through a calibrated dropper and into a receiving test tube as described by Rector *et al.* (1966) in their studies with mineral spirits. High-pressure air forced through a spray nozzle created negative pressure that drew the liquid and room air from the receiver up through the nozzle where it was vaporized. The contaminant then traveled up the laminar-flow tube, into the drum-type impactor and through a Venturi tube to the incoming air-supply line where the contaminant was diluted and mixed immediately prior to entering the exposure chamber.

The gases received in cylinders (ammonia and dimethylamine) were controlled by adjusting regulators and valves to yield the desired input levels as indicated by an appropriate flowmeter. The negative pressure at the spray nozzle, created by a stream of high-pressure air, drew room air into the mixing bottle which diluted the metered stream of gas arriving from the cylinder. The diluted gas then followed a route identical to that described above for vaporized liquids.

Contaminant Analysis

Ammonia. Nominal input concentrations were used in the 40 and 155 mg/m^3 exposures. In all other runs, a dispersive double-beam infrared spectrophotometer[3] fitted with a variable-pathlength gas cell was used to monitor the chamber concentrations. Samples of chamber atmosphere were continuously drawn through the cell, and the percent transmission was recorded at a wavelength of 10.34 μ.

Formaldehyde. Formaldehyde concentrations were monitored with a nondispersive infrared analyzer equipped with a catalytic oxidizer.[4] This instrument is designed to monitor indirectly organic compounds in an air stream after oxidizing them to CO_2

[2] Ammonium hydroxide, ethylene glycol, absolute ethanol, paraformaldehyde; Fisher Scientific Corp., Fair Lawn, New Jersey; gaseous ammonia and dimethylamine; Matheson Company, Inc., East Rutherford, New Jersey.

[3] Model 21 Infrared Spectrophotometer, Perkin Elmer Corp., Norwalk, Connecticut.

[4] Model 200 LIRA Mine Safety Appliance Co., Pittsburgh, Pennsylvania.

and is equipped with a system of differential optics that automatically subtracts background CO_2.

Dimethylamine. The dimethylamine concentration was continuously monitored with a hydrogen flame-ionization detector[5] which measures total hydrocarbon content. Although actual contaminant concentration in the chamber was too low to permit direct sampling with this instrument, an alternate method of sampling from the drum impactor prior to dilution was utilized. The readings obtained were compared with standards, and actual chamber concentrations were calculated.

Ethanol. The ethanol exposure was monitored with a gas chromatograph.[6] Nitrogen was used as a carrier for the samples through a 6-ft column (¼ inch o.d.) at 69°C packed with 5% Carbowax 1500 on Halaport F.[7] The chamber atmosphere was sampled every 30 minutes by an automatic sampling valve which injected a 10 ml sample into the column.

Ethylene Glycol. Atmospheric samples from the chamber were collected in water and then oxidized to formaldehyde with periodic acid, and concentrations were determined using modified Schiff's reagent following the method described by Jacobs (1949).

Biochemical Methods

In these screening experiments, a limited number of biochemical and histochemical determinations were carried out. Serum urea nitrogen concentration was determined by an automated method based on the work of Marsh *et al.* (1957). The activities of serum aspartate aminotransferase (EC 2.6.1.1)[8] and alanine aminotransferase (EC 2.6.1.2) were measured by the colorimetric method described by Reitman and Frankel (1957), serum alkaline phosphatase (EC 3.1.3.1) by a method based on the work of Bessey *et al.* (1946), and serum lactate dehydrogenase (EC 1.1.1.27) by the Berger and Broida method.[9] Histochemical detections of reduced nicotinamide adenine dinucleotide (NADH); reduced nicotinamide adenine dinucleotide phosphate (NADPH); succinate (EC 1.3.99.1), lactate (EC 1.1.1.27), isocitrate (NADP) (EC 1.1.1.42), and β-hydroxybutyrate (EC 1.1.1.30) dehydrogenases in liver and kidney specimens were carried out and roughly quantitated on a 0 to 4+ basis, using the method described by Ballogh *et al.* (1961).

Experimental Animals

Five species of animals were exposed to the test materials. These were male and female Sprague-Dawley [NMRI: O(SD)] and Long-Evans [NMRI: (LE)]-derived rats, male and female Princeton-derived guinea pigs [NMRI: (ASH)], male New Zealand albino rabbits (ROW: NZW), male squirrel monkeys (*Saimiri sciureus*), and purebred male beagle dogs. A typical loaded chamber contained 15 rats, 15 guinea

[5] Model 223 Perkin Elmer, Perkin Elmer Corp., Norwalk, Connecticut.
[6] Model A110 Chromalab, Glowall Corp., Willow Grove, Pennsylvania.
[7] F & M Scientific Corp., Avondale, Pennsylvania.
[8] The nomenclature and numerical classification of the enzymes studied are in accordance with the "Report of the Commission on Enzymes of the International Union of Biochemistry, 1961" published by Pergamon Press, New York, 1961.
[9] "The Colorimetric Determination of Lactic Dehydrogenase in Serum and Other Fluids at 400 to 455 mμ." Technical Bulletin No. 500. Sigma Chemical Co., St. Louis, Missouri.

pigs, 3 rabbits, 3 monkeys, and 2 dogs. In 3 of the ammonia experiments only rats were used because it appeared from previous exposures that these animals were the most susceptible.

Each animal received the appropriate commercially prepared dry food, and all animals except guinea pigs were given water ad libitum. The guinea pigs and rabbits received a supplement of 1/4 head lettuce per day; the dogs received a supplement of meat-based canned dog food, and the monkeys received oranges, bananas, and hard-boiled eggs. Control animals were maintained in dynamic chambers without contaminant but otherwise were handled in a manner identical to the experimental animals.

Blood samples were taken before and after the exposures for the determination of hemoglobin concentration, packed erythrocyte volume as expressed by the microhematocrit, and total leukocyte counts. All animals were routinely checked for visible signs of toxicity, such as alterations in behavior, physical appearance, breathing pattern, and locomotor activity. At the termination of each experiment, animals were sacrificed with an overdose of pentobarbital and necropsied. Sections of heart, lung, liver, kidney, and spleen were retained for histopathologic examination from approximately half of the surviving guinea pigs and rats and from all the surviving monkeys, dogs, and rabbits in each experiment. In addition, sections of brain, spinal cord, and adrenals were retained from monkeys and dogs as well as thyroid tissue from the dogs.

RESULTS

Repeated Ammonia Exposure (155 mg/m³)

There were no deaths (Table 1) or visible signs of toxicity in any of the animals. Hematologic values were within our normal limits,[10] and there were no gross abnormalities seen in organs or tissue at necropsy. Histopathologic examination showed evidence of focal pneumonitis in the lung of 1 of the 3 monkeys; no other changes were noted.

Repeated Ammonia Exposure (770 mg/m³)

There were no deaths during the exposure, but mild to moderate lacrimation and dyspnea were evident in the rabbits and dogs during the first week. During the second week, these signs disappeared and no further indications of irritation or toxicity were noted. Hematologic results and gross observations at necropsy were not significant, and histopathologic examination did not reveal any changes that could be definitely attributed to the exposure. The lungs of the rats and guinea pigs showed rather consistent nonspecific inflammatory changes that were more extensive than those seen in control animals.

[10] Normal NTU hematologic values:

	Leukocytes (10^3/mm³)	Hemoglobin (g/100 ml)	Hematocrit (%)
Rats	16.5 ± 4.7	1[illegible] ± 1.2	47 ± 4
Guinea pigs	5.9 ± 1.4	14.9 ± 1.1	47 ± 3
Rabbits	9.3 ± 2.7	12.8 ± 1.1	40 ± 4
Monkeys	9.7 ± 3.3	14.2 ± 1.3	43 ± 4
Dogs	14.2 ± 3.9	15.2 ± 1.4	47 ± 4

TABLE 1

MORTALITY IN ANIMALS EXPOSED TO SELECTED TRACE CONTAMINANTS

Material	Concentration (mg/m^3)[a]	Type of study[b]	Number died/number exposed				
			Rat	Guinea pig	Rabbit	Dog	Monkey
Ammonia	155 ± 32	R	0/15	0/15	0/3	0/2	0/3
	770 ± 55	R	0/15	0/15	0/3	0/2	0/3
	40 ± 2	C[c]	0/15	0/15	0/3	0/2	0/3
	127 ± 8	C	0/48	—	—	—	—
	262 ± 10	C	0/49	—	—	—	—
	455 ± 23	C[d]	50/51	—	—	—	—
	470 ± 16	C	13/15	4/15	0/3	0/2	0/3
Ethylene glycol	10 ± 1	R	0/15	0/15	0/3	0/2	0/2
	57 ± 14	R	0/15	0/15	0/3	0/2	0/2
	12 ± 2	C	1/15	3/15	1/3	0/2	0/3
Formaldehyde	4.6 ± 0.4	C	1/15	0/15	0/3	0/2	0/3
Dimethylamine	9 ± 1	C	0/15	0/15	0/3	0/2	0/3
Ethanol	86 ± 7	C	0/15	0/15	0/3	0/2	0/3
Control	—	C	4/123	0/73	0/12	0/12	0/8

[a] Mean ± SD.
[b] R = 30 repeated exposures, 8 hr/day, 5 days/week; C = continuous 90-day exposure.
[c] 114-day continuous exposure.
[d] Terminated on 65th day.

Continuous Ammonia Exposure (40 mg/m³)

This experiment differed from the other continuous exposures in that it was run for 114 instead of 90 days. There were no deaths or signs of toxicity in any of the animals. Observations at necropsy were normal, and subsequent histopathologic examination revealed lipid filled macrophages in the lungs of both dogs, 1 monkey, and 1 rat although these were probably of no clinical significance. No lung alterations were seen in the remaining experimental and control animals.

Continuous Ammonia Exposure (127 mg/m³)

There were no deaths or signs of toxicity in any of the 48 rats exposed, and all hematologic values were normal. There were no gross lesions or other abnormalities in any of the organs or tissues. Microscopic examination revealed nonspecific inflammatory changes in the lungs and kidneys from approximately 50% of the experimental and control animals examined. No specific chemically induced changes were seen. The histochemical results of the determination of NADH and NADPH and succinate, isocitrate, lactate, and β-hydroxybutyrate dehydrogenases in liver specimens did not show any significant differences between experimental and control rats.

Continuous Ammonia Exposure (262 mg/m³)

No mortality or pronounced signs of toxicity were noted in any of the 49 rats exposed, although approximately 25% of the animals had mild nasal discharge. Hematologic results were essentially normal although 4 rats had slightly elevated leukocyte counts. There were no gross lesions observed during the necropsies. The

tissue examined microscopically showed nonspecific circulatory and degenerative changes in lungs and kidneys that were difficult to relate specifically to ammonia inhalation.

Continuous Ammonia Exposures (455 mg/m³; 470 mg/m³)

In the 455 mg/m³ run, 32 of 51 rats died by day 25 of exposure, and 50 by day 65, when the experiment was terminated. All showed mild signs of dyspnea and nasal irritation. No histopathologic examinations were made on these animals.

In the 470 mg/m³ exposure, 13 of 15 rats and 4 of 15 guinea pigs died. Marked eye irritation was noted in dogs and rabbits. This was exemplified by heavy lacrimation in the dogs and erythema, discharge, and opacity over 1/4 to 1/2 of the cornea in the rabbits. The dogs also had nasal discharge. Hematologic values did not differ significantly from the controls.

Observations at necropsy indicated moderate lung congestion in 2 rabbits and a hemorrhagic lesion in the lung of 1 dog. Histopathologic examination revealed consistent lung involvement as evidenced by focal or diffuse interstitial pneumonitis in all animals examined. In addition, there was calcification of renal tubular and bronchial epithelia, proliferation of renal tubular epithelium, myocardial fibrosis, and fatty changes of the liver plate cells in several animals of each species. Control animals showed similar changes but of lesser severity.

Repeated Ethylene Glycol Exposure (10 mg/m³)

There were no deaths during the exposure. Mild conjunctivitis was noted in 1 eye of each of 2 rabbits during the 4th and 5th weeks, which persisted until the end of the exposure; each of these rabbits also developed a small lesion over the irritated eye. These signs were probably brought on by accidental trauma which may have been aggravated by the exposure. Hematologic values of all animals were within normal limits. Histopathologic examination revealed mild congestion in the spleens of both dogs; hepatic fatty changes in 2/8 guinea pigs and 1/8 rats; and focal necrosis in the liver of 1/8 guinea pigs and 1/8 rats. Focal necrosis of the liver was also seen in 1/3 control guinea pigs.

Repeated Ethylene Glycol Exposure (57 mg/m³)

There were no deaths and no signs of toxicity during the exposure. All hematologic data and observations during necropsies compared favorably with controls. Histopathologic examinations revealed nonspecific inflammatory changes in the lungs and occasionally the hearts of all species. The livers of 2 of the 3 monkeys and 1 of the 8 guinea pigs revealed areas of focal necrosis; these were not considered to be chemically induced. Serum urea nitrogen concentrations in the experimental guinea pigs of 24 ± 3 mg/100 ml were not significantly different from the control values of 21 ± 3 mg/100 ml.

Continuous Ethylene Glycol Exposure (12 mg/m³)

Exposure at this concentration caused moderate to severe eye irritation in the rabbits and rats. Erythema, edema, and discharge began in rabbits after 3 days of exposure, the edema being severe enough to result in virtual closure of the eyes. Two rats

developed corneal opacity after 8 days and appeared to be blind for the remainder of the exposure. One rabbit, 3 guinea pigs, and 1 rat died during exposure although they had not shown any specific signs of toxicity. All hematologic data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathologic examination showed inflammatory changes in the lungs of all species and to a lesser degree in controls. Occasional foci of inflammatory cells were seen in kidneys from several guinea pigs, and 1 rabbit had hamartomatosis in liver bile ducts. These, however, were not interpreted as being specific chemically induced changes. Serum aspartate aminotransferase activity, expressed in Karmen (1955) spectrophotometric units, ranged from 20 to 26 units in the dogs, 34 to 41 units in the guinea pigs, and 46 to 50 units in the rabbits. The range of serum alanine aminotransferase activity, expressed in the same units was 14 to 29 for the dogs, 17 to 33 for the guinea pigs and 32 to 42 for the rabbits. Alkaline phosphatase activities, in micromoles of *p*-nitrophenol liberated, ranged from 0.6 to 0.7 in the dogs, 1.5 to 2.3 in the guinea pigs, and 0.8 to 1.9 in the rabbits. The activities of lactate dehydrogenase, expressed in the spectrophotometric units of Wroblewski and LaDue (1955), were found to be 116 to 120 in the dogs, 115 to 120 in the guinea pigs and 310 to 350 in the rabbits. All the values are considered to be within normal limits for these species and strains. Histochemical studies of succinate, lactate, isocitrate, glucose 6-phosphate, and β-hydroxybutyrate dehydrogenases in liver and kidney tissue revealed no significant differences between experimental and control animals.

Continuous Formaldehyde Exposure (4.6 mg/m³)

One of the 15 rats died; none of the other animals showed any signs of illness or toxicity. Hematologic values were also normal. On histopathologic examination, the lungs of all species consistently showed varying degrees of interstitial inflammation, and the hearts and kidneys from guinea pigs and rats showed focal chronic inflammatory changes. It was uncertain whether these changes were caused by the formaldehyde inhalation.

Continuous Dimethylamine Exposure (9 mg/m³)

There were no deaths or signs of toxicity, and all hematologic values were normal. On histopathologic examination, interstitial inflammatory changes were noted in the lungs of all species. The 3 rabbits and 2 of the 3 monkeys showed dilatation of the bronchi. Specific chemically induced histopathologic changes, however, were not noted.

Continuous Ethanol Exposure (86 mg/m³)

There were no deaths or signs of toxicity, and all hematologic values were within normal limits. Histopathologic examination revealed nonspecific circulatory and inflammatory changes that were not considered to be chemically induced.

DISCUSSION

One of the objectives in presenting preliminary data of this nature is to make available information which may be useful in establishing or supporting Threshold Limit

Values (TLV) or Confined Space Guidelines (CSG). The CSG is the concentration limit suggested for confined systems where humans may be exposed continuously for up to 90 days.

Ammonia

The TLV for ammonia has been set by the Committee on Threshold Limit Values (1966) at 35 mg/m³. Weatherby (1952) exposed male guinea pigs to 119 mg/m³, approximately 3 times the TLV, for 6 hr/day, 5 days/week for 18 weeks. He found no adverse effects in guinea pigs sacrificed after 12 weeks, but found mild, though definite changes in the spleen, kidney, adrenals, and liver of animals exposed 18 weeks. Congestion and early degenerative changes were present in these organs. In the repeated studies reported here, exposures were made at approximately 5 and 22 times the TLV. At 5 times the TLV exposure, there was no evidence of adverse effects. However, at 22 times the TLV, there were signs of ocular and nasal irritation and an increased incidence of and more extensive diffuse interstitial pneumonitis than seen in controls.

In the continuous exposures, experiments were carried out at 2, 7, 15, and 25 times the level of 18 mg/m³ suggested as a CSG. At 2 and 7 times the CSG, the parameters of hematology and histopathology were considered to be within normal limits. When rats were exposed to 15 times the CSG, mild nasal discharges and slightly elevated leukocyte counts were noted in a number of the animals; these leukocyte elevations were probably due to infection and not to the exposure. There were also nonspecific circulatory and degenerative changes in lungs and kidneys. The high mortality and the ocular and nasal irritation noted during the 2 exposures at approximately 25 times the CSG, coupled with an increase in pathologic findings in surviving animals, would indicate that the effects were due to the exposure.

Ethylene Glycol

Flury and Wirth (1933) found that rats exposed to 500 mg/m³ ethylene glycol for 28 hours during 5 days developed slight narcosis. Wiley *et al.* (1936) exposed mice and rats to 350 to 400 mg/m³ for 8 hours per day for 16 weeks without producing adverse effects. It has been reported[11] that no ill effects were seen in humans exposed continuously for 4 weeks to a wet spray of ethylene glycol at a concentration of 17 mg/m³, but that men would not remain in an atmosphere of 50 mg/m³ for more than a brief period of time. Renal injury[11] but no ocular damage, in a chimpanzee exposed for a prolonged period to 265 mg/m³ ethylene glycol as an aerosol has also been reported.

Rats, guinea pigs, and rabbits were exposed continuously to approximately 12.6 mg/m³ ethylene glycol[12] in order to determine the degree and type of eye damage. After 47 days, none of the 30 rats or 20 guinea pigs showed corneal changes. Four rabbits were exposed for 17 days and observed daily the first week for corneal alterations. There was minimal cloudiness of the surface layer of the corneal epithelium during the first 3 days but no alterations were seen thereafter.

In an additional test, a 10% solution of ethylene glycol in water and concentrated ethylene glycol were each instilled in 1 eye of 2 rabbits. After a 7-day observation

[11] Committee on Toxicology, NAS—NRC, minutes of the Annual Meeting, January 31, 1969.

[12] J. D. MacEwen, personal communication from SysteMed Corp., Toxic Hazard Research Unit, Dayton, Ohio.

period, the corneas of the treated eyes were taken for histopathologic examination. No significant corneal pathology was noted.

In our repeated exposure at 57 mg/m^3, none of the animals showed any signs of ocular or nasal irritation. The eye signs noted in the 10 mg/m^3 run were probably due to accidental damage, not to the exposure.

In the continuous exposure at 12 mg/m^3 there was moderate to severe eye irritation in all 3 rabbits and corneal opacity, with apparent blindness, in 2 of the 15 rats. Both species were affected within 8 days of the initial exposure.

No TLV has been set for ethylene glycol. The Committee on Toxicology of the National Research Council[13] has recommended a guideline of 22 mg/m^3 for exposure to vapors over a 2-week continuous period in space flight. In view of the eye irritation observed in rabbits and rats after only 8 days at 12 mg/m^3, the level of 22 mg/m^3 set for 2-week space flights may be too high.

Formaldehyde, Dimethylamine, and Ethanol

Since only one experiment each was made for formaldehyde, dimethylamine, and ethanol, there are insufficient data to apply to a CSG. Most parameters were essentially normal in the continuous 90-day exposure to formaldehyde at 4.6 mg/m^3; however, the death of 1/15 rats indicates the need for additional studies. The continuous exposure to dimethylamine at 9 mg/m^3 produced mild inflammatory changes in the lungs of all species and dilated bronchi in 3 of 3 rabbits and 2 of 3 monkeys. All parameters studied were normal in the continuous exposure to ethanol at 86 mg/m^3.

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[13] Committee on Toxicology, NAS—NRC, letter report of July 30, 1965.

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